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Phase II of carotenoid bandshift is mainly due to the electrogenic protonation of the secondary quinone acceptor

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The flash-induced kinetics of transmembrane electric potential ($\Delta\psi$) generation with a rise-time of 0.1–0.2 ms were investigated in *Rhodobacter sphaeroides* chromatophores by electrometry and by monitoring the electrochromic absorbance changes of carotenoids. An analysis of the results obtained electrometrically shows that this electrogenic phase is observed only after even-numbered flashes. The sensitivity of this phase to *o*-phenanthroline, the flash-number dependence of its amplitude and the decrease of its rise-time with decreasing pH indicate that it is due to the dismutation of Q_A^- and Q_B^- and to subsequent protonation of a doubly reduced ubiquinone Q_B molecule. The phase of the carotenoid bandshift with $\tau \sim 0.1$ ms (the so-called phase II) was shown to be sensitive to *o*-phenanthroline, with its rise-time decreasing with decreasing pH. It is concluded that a considerable part of phase II of the electrochromic carotenoid changes with $\tau \sim 0.1$ ms is caused by the electrogenic reaction $Q_A^-Q_B^- + 2H^+ \rightarrow Q_AQ_BH_2$, but not solely by the oxidation of cytochrome c_2, as proposed earlier [(1973) Biochim. Biophys. Acta 325, 102–113].

Electrochromic absorbance change; Carotenoid; Electron transfer; Reaction center; (*Rhodobacter sphaeroides*)

1. INTRODUCTION

The flash-induced electrochromic changes of carotenoids in chromatophores consist of three phases [1–4]. It has been established that phase I is associated with charge separation between P870 and Q_A. It disappears under oxidizing conditions at $E_h \sim 500$ mV and under reducing conditions at $E_h \sim -100$ mV [2–4]. In reductive redox-titration of electrochromic changes at $E_h \sim 300$ mV, a second phase appears (phase II, $\tau \sim 0.15$ ms) which contributes about 30% to phase I. Since this phase titrates with $E_{m7} \sim 300$ mV and its rise-time correlates well with the time of cytochrome c_2 oxidation by P870, one can assume that phase II is solely due to the electrogenic reaction of electron transfer from cytochrome c_2 to P870 [2–4]. Furthermore, a slower phase of the electrochromic changes of carotenoids (phase III, $\tau \sim 1$–20 ms), which is associated with the electrogenic reactions of the cytochrome bc_1 complex, is observed [1–5]. This phase is partly inhibited by antimycin A. Myxothiazol completely inhibits this phase [6].

At the same time kinetic measurements of $\Delta\psi$ generation in chromatophores show that the reduction of Q_B^- to Q_BH_2 gives rise to an electrogenic phase with $\tau \sim 0.1$ ms at pH 7, which contributes as much as 10–20% to the total $\Delta\psi$ generated by the flash [7–9].

Here we show that phase II of the electrochromic changes with $\tau \sim 0.1$–0.2 ms is mainly

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Abbreviations: RC, reaction center; P870, reaction center bacteriochlorophyll dimer; Q_A, Q_B, primary and secondary quinone acceptors of RC; $\Delta\psi$, transmembrane electric potential difference; TMPD, *N,N,N',N'*-tetramethyl-*p*-phenylenediamine; Mes, 4-morpholineethanesulphonic acid

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caused by the electrogenic protonation of the secondary quinone acceptor of RC.

2. MATERIALS AND METHODS

Asolectin (phosphatidylcholine type II-S), TMPD and ubiquinone (Q-10) were from Sigma, the buffers and antimycin being obtained from Serva. Myxothiazol was kindly provided by Professor W. Trowitzsch.

Photosynthetic bacteria (*Rhodobacter sphaeroides* wild type) were grown for 4–5 days under anaerobic conditions at 30°C under constant illumination in an Ormerod medium. Chromatophores were isolated from washed cells by sonication [7,9]. Kinetic measurements of $\Delta\psi$ generation have been described in [7–9]. Rapid absorption kinetics were measured with a Soviet single-beam spectrophotometer. Saturating light pulses were delivered from a Lomo OGM-40 ruby laser (λ = 694 nm, pulse half-width, 20 ns). The data storage and processing system included a DL-1080 (Data Lab) transient recorder interfaced to a Nova-3D mini-computer (Data General).

Q_B function in collodion film-associated chromatophores was reconstituted by adding Q-10 (20 mg/ml) to the solution of asolectin in decane used to impregnate the collodion film in the measuring cell, as described in [8].

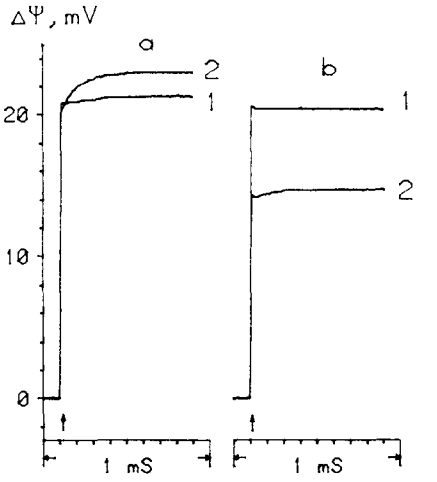

Fig.1. Photoelectric responses of *Rhodobacter sphaeroides* chromatophores induced by the first (1) and second (2) flash in the absence (a) and presence (b) of 3 mM *o*-phenanthroline. Incubation medium: 30 mM Mes (pH 6.5), 30 μM TMPD, 1 mM potassium ferrocyanide. E_h = 300 mV. Arrows here and further indicate laser flash excitation. 5 μM antimycin A and 3 μM myxothiazol were present.

3. RESULTS AND DISCUSSION

Fig.1 shows typical photoelectric responses of chromatophores induced by a first (curve 1) and second (curve 2) flash in the presence (b) and absence (a) of *o*-phenanthroline, an inhibitor of electron transfer from the primary to secondary quinone acceptor. Upon the second flash, an additional electrogenic phase was observed with a characteristic time of ~0.1 ms. *o*-Phenanthroline inhibits this phase. As seen from fig.2, the rise-time of this additional phase decreases upon acidification. The sensitivity of this phase to *o*-phenanthroline and the decrease of its rise-time with decreasing pH indicate that it is due to the dismutation of Q_A^- and Q_B^- with subsequent protonation of a doubly reduced ubiquinone molecule [7–9]:

$$Q_A^-Q_B^- + 2H^+ \longrightarrow Q_AQ_BH_2 \qquad (1)$$

The large amplitude of the 0.1 ms phase of $\Delta\psi$ observed after the second flash poses the problem of recording this phase by observing electrochromic changes of carotenoids. Sinced the signal/noise ratio for optical measurements is essentially lower than that for the electrometric method used by us, observation of the electrogenic phase corresponding to reaction 1 requires averaging of several measurements. The measurement of the carotenoid bandshift was made under conditions when about half of the RCs contained the secondary quinone in the semiquinone form before the flash.

Fig.3 shows typical traces of the flash-induced carotenoid bandshift. Phase II with a rise-time of ~0.1 ms (pH 6.5) is observed, in addition to the fast phase (τ < 100 ns) (curve a). The former is completely inhibited by *o*-phenanthroline (curve b). The rise-time of this phase depends on pH. This dependence agrees well in behavioral pattern with the pH dependence of the rise-time of the photoelectric response observed electrometrically after the second flash (fig.4).

There is every reason to conclude that at least in ultrasonically isolated chromatophores phase II of the carotenoid bandshift with τ ~ 0.1–0.2 ms is mainly the result of the electrogenic reaction 1.

The evidence that phase II of the carotenoid bandshift is entirely due to the electrogenic oxidation of cytochrome c_2 is based on the coincidence of the kinetics of cytochrome c_2 oxidation by P870 and of phase II and on the fact that the amplitude of phase II increases with a decrease in the redox-potential below 300 mV, as reported in the

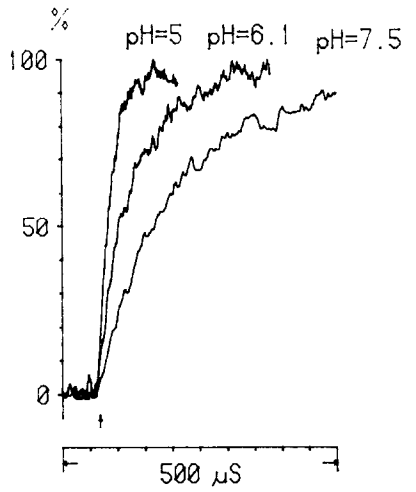

Fig.2. The effect of pH on the kinetics of the $\Delta\psi$ generation phase with $\tau \sim 0.1$–0.2 ms, induced by the second flash. Each curve is derived by subtracting the curve induced by the second flash from that induced by the first flash and normalized to 100%.

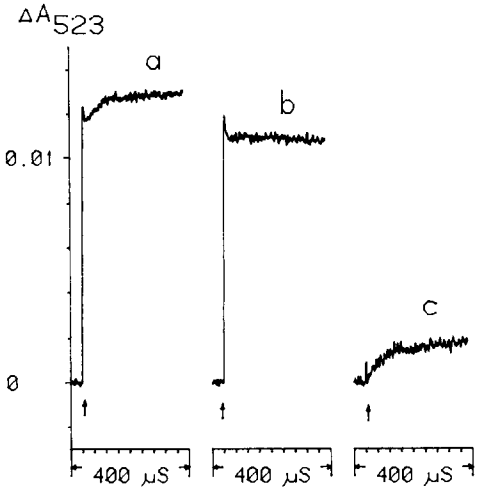

Fig.3. Absorbance changes at 523 nm induced by a laser flash in *Rhodobacter sphaeroides* chromatophores, reflecting the carotenoid bandshift in the absence (a) and presence (b) of 2 mM *o*-phenanthroline. Incubation medium, as in fig.1. Each curve is derived by averaging of 64 curves.

literature [2–4]. The characteristic time of 0.1–0.2 ms may, however, correspond to both cytochrome c_2 oxidation [4,10] and the electron-transfer reaction between the quinone acceptors [11,12]. Besides, a similar E_h dependence of the phase II amplitude may indirectly be related to c_2 functioning, as shown earlier by us [13,14]. In fact, under oxidizing conditions ($E_h \sim 400$ mV) only P870 is reduced. Therefore, following light activation, only one electron can be transferred to Q_B with the result that the quinone may be reduced only to the semiquinone species. As shown earlier [7–9,15,16], the reaction $Q_A^- Q_B \longrightarrow Q_A Q_B^-$ is not electrogenic. Thus, under these conditions the phase with a $\tau \sim 0.1$–0.2 ms, corresponding to electrogenic reaction 1, is not observed. On decreasing the redox potential, cytochrome c_2 is reduced. This leads to a rapid reduction of P870 photooxidized by the measuring beam with the result that the electron cannot return to P870. The secondary quinone may exist in the semiquinone species for several minutes in the absence of acceptors. Hence, it is the equilibrium reduction of cytochrome c_2 (or another electron donor for P870) that is necessary for the electrogenic phase to occur.

As seen from fig.3, the relative amplitude of the *o*-phenanthroline-sensitive phase is about 10% of that of the electrogenic phase associated with charge separation between P870 and Q_A. With our preparation, the electrogenic stage related to cytochrome c_2 oxidation is not observed, probably because of the loss of cytochrome c_2 during

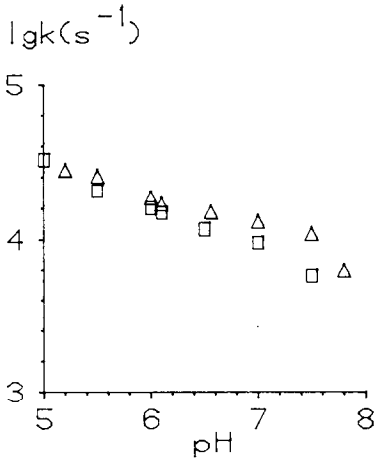

Fig.4. The effect of pH on the logarithm of the rate constant ($k = 1/\tau$) for $\Delta\psi$ generation induced by the second flash (△) and the *o*-phenanthroline-sensitive phase of carotenoid bandshift (□). Incubation medium, as in fig.1. Buffers used for varying pH: Mes (pK 6.2), Mops (pK 7.2), Hepes (pK 7.5) at 20 mM.

ultrasonic treatment of the bacterial cells [4]. At the same time, the known topography of RC [17,18] and the results of kinetic [4,19] and electrometric [20] measurements indicate that the electrogenic phase arising from the reaction $c_2 \longrightarrow$ P870 does exist.

The obvious and most attractive conclusion is that on the microsecond time scale there exist at least two electrogenic phases, one caused by the quinone reaction (eqn 1), which is *o*-phenanthroline-sensitive, and the other, which is *o*-phenanthroline-insensitive, caused by cytochrome c_2 oxidation by $P870^+$. Their relative amplitudes depend on the preparation procedure, redox potential, measuring beam intensity, donor and acceptor concentrations and other factors.

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